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(54) **Treatment of infertility.**

(57) The use of somatotrophin releasing factor (GRF) is disclosed for the treatment of infertility. Also disclosed for such treatment is the use of somatotrophin releasing factor along with gonadotrophins, particularly, follicle stimulating hormone (FSH) or preparations containing FSH. Pharmaceutical preparations containing somatotrophin releasing factor, including preparations containing both somatotrophin releasing factor and follicle stimulating hormone, are also disclosed.

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TREATMENT OF INFERTILITY

Summary of the invention

The invention refers to the new use of GRF (Somatotrophin releasing factor), alone or in combination with the follicle-stimulating hormone, in the treatment of infertility.

The present invention relates to the treatment of male and female infertility using a Somatotrophin releasing factor, alone or in combination with the follicle-stimulating hormone, or to the relevant pharmaceutical preparations.

The efficacy of the Follicle Stimulating Hormone (FSH) in the induction of Progesterone synthesis by ovarian granulosa cells is well known. The acquisition of the capacity to synthetize progesterone by such cells, in turn, determines the completion of the maturation of the oocyte, for correct follicle growth and the normal functioning of corpus luteum.

Therapy with FSH and Chorionic Gonadotrophin has become the main treatment of anovulatory sterility due to insufficient endogenous secretion of gonadotrophins. FSH can be administered as Menotrophin or HMG (Human Menopausal Gonadotrophin), a preparation containing the same number of units of biological activity (I.U.) of the hormones FSH and LH (= Luteinizing Hormone), or as Urofollitrophin, a preparation containing FSH and insignificant quantities of LH.

The applicant markets such preparations under the names Pergonal$^{(R)}$ and Metrodin$^{(R)}$, respectively.

Due to the demonstrated efficacy of gonadotrophins on the Sertoli cells, that is, the male equivalent of the ovarian granulosa cells, the same preparations are also used for the treatment of male infertility.

As stated above, while treatment with gonadotrophins has become the treatment of choice in female and male infertility, there are some clinical conditions in which this kind of treatment is not efficacious.

Studies carried out in normal subjects during the menstrual cycle have demonstrated that the intravenous administration of GRF produces an increase in serum levels of HGH and Sm-C but not of prolactin, LH or FSH (Evans W.S., et al. - Effects of human pancreatic growth-hormone-releasing factor-40 on serum growth hormone, prolactin, luteinizing hormone, follicle-stimulating hormone and somatomedin-C concentrations in normal women throughout the menstrual cycle. J. Clin. Endoc. Metab. 59: 1006, 1984).

It has also been demonstrated that Sm-C in nanomolar concentrations enhances FSH capacity in inducing synthesis of progesterone by cultured rat granulosa cells (Adashi E.Y., and others - Somatomedin-C synergizes with follicle- stimulating hormone in the acquisition of progestin biosynthetic capacity by cultured rat granulosa cells. Endocrinology 116:2135, 1985).

Not all the growth factors carry out the same kind of action.

It is known, for example, that EGF (= Epidermal Growth Factor) has an inhibitory effect on FSH-induced cyclic AMP production, and therefore on ovarian granulosa cells differentiation. Another growth factor, TGF-beta (= Transforming Growth Factor-beta) modulates the effect of FSH and EGF during the differentiation process of the same cells (Pei Feng, and others - Transforming Growth Factor beta regulates the Inhibitory Actions of Epidermal Growth Factor during Granulosa Cell Differentiation - J. Biol. Chem. 261(30), 14167, 1986).

In conclusion, it is evident that growth factors have a regulatory effect on the above mentioned processes of cell differentiation and interact with FSH at the cell target level of this gonadotrophin. Nevertheless, their mechanism of action and relative synergism have not yet been explained.

It has now been discovered that GRF is capable of interacting with the ovarian follicle-genesis and spermatogenesis regulatory mechanisms and with the maturation of spermatozoa.

It has also been discovered that GRF increases the in-vivo effect of gonadotrophins on the gonads.

Therefore, the main object of the present invention is the therapeutic use of GRF in treatment of cases of female and male infertility. A further object of this invention consists in a combined therapy of GRF and gonadotrophins, applicable whenever it is desirable or necessary to increase the effect of gonadotrophins on the gonads.

Other aims and advantages of the present invention will result from the following detailed description.

Abundant literature on GRF exists. (See, for example, Guillemin et al. Science 218, 585, 1982; Esch et al., J. Biol. Chem. 258,1806, 1983).

Various forms of GRF have been purified and their aminoacid sequence has been determined. GRF-44 contains the complete aminoacid sequence of GRF-40 and is extended at the carboxylic terminal by four aminoacids.

GRF-40, in turn, contains the complete aminoacid sequence of GRF-37 and is extended at the carboxylic terminal by three aminoacids

It has been shown that peptide GRF-29 is also biologically active (J. Rivier et al., Nature, 300, 276-8, 1982).

It has also b en d monstrated that GRF-NH_2-44 is the mature hormone and that GRF 40, 37 and 29 are all biologically active fragments.

It has further been demonstrated that the various GRFS react both at the level of synthesis and at the level of release of the growth hormone by the hypophysis (Brazeau et al., Proc. Nat. Acad. Sci. 79, 7909, 1982; Baringa

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et al., Nature 306, 84, 1983).

GRF can be administered by the intravenous, intramuscular or subcutaneous routes. Other routes of administration, capable of providing the necessary haematic levels of GRF, are included in the present invention.

In the clinical trials reported below, GRF-29 was used in the form of lyophilized ampoules containing 100 or 150 micrograms (mcg) of GRF and 10 milligrams (mg) of mannitol as excipient. The lyophilized substance, dissolved in 2 ml of physiologic saline, was administered by the intravenous route.

The pharmaceutical production of GRF ampoules was carried out by using traditional methods and does not present any particular difficulty.

IN-VITRO STUDIES

Studies have been carried out to determine immunoreactivity to GRF in human seminal and follicular fluids and in the supernatant of isolated Leydig rat cells; furthermore, binding sites for GRF have been identified in isolated Sertoli rat cells.

1. MATERIALS AND METHODS

Follicular Fluid (F.F.)

Follicular fluid was obtained by means of aspiration of the follicles during the laparoscopy carried out under the GIFT or the FIVET programme. The follicular fluid was immediately centrifuged for 15 minutes at 2000 rpm to remove cells and cell fragments, and the supernatant was frozen at -20°C, until its extraction for the GRF dosage.

Seminal Fluid (S.F.)

Seminal fluid was collected from healthy volunteers, ranging in age from 24 to 36, by means of masturbation after 2-5 days of sexual abstinence. After collection, the seminal fluid was kept at room temperature for about 20 minutes until the coagulum liquified and then it was centrifuged for 15 minutes at 1500 rpm to remove spermatozoa and other cellular species. The supernatant was diluted 1:1 with 0.5 M acetic acid solution, boiled for 15 minutes and centrifuged for 10 minutes at 1000 rpm. The supernatants were frozen at -20°C.

Leydig Rat Cells Supernatants

Sprague Dawley adult rats aged 50 days were sacrificed, and their testicles removed, decapsulated and placed in a culture medium (Earle) containing 0.025% of collagenase.
After 15-20 minutes of incubation at 34°C under stirring, the scattered cells were separated from the tubules, centrifuged and replaced in a culture medium containing 0.025% of collagenase.
After three hours of incubation, the supernatant was separated and frozen at -20°C.

Extraction procedure

The samples prepared as described above were thawed and passed through octadodecasilillsilice columns (Sep-Pak C18, Waters), activated with 90% methanol and 0,1% BSA; the material was eluted with 90% methanol in 1% HCOOH and subsequently dried under a nitrogen flow at room temperature. Recovery of labelled GRF as extracted by this method is 70%.
The dried residue is reconstituted in PBS + 0,3% BSA, 10 mM EDTA and dosed with the RIA method.

RIA Dosage of GRF

Label used: (3-[I]^{125} iodotyrosil^{10}) human GRF-44 (Amersham), specific activity about 2000 Ci/mmol.
Anti-serum: anti-GRF obtained in the rabbit (Amersham) against human GRF-44. Cross-reactivity 100% with GRF-29.
Cold: human GRF-44 (Sigma).
Incubation volume: 24 h at 4°C.
Free separation from ligand: with carbon dextran.
Sensibility of the method: 7,8 pg/tube.
Intra- and inter-dosage variability: 5% and 10% respectively.

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Preparation of Sertoli Cells and GRF Binding Conditions

Sertoli cells were isolated from the testicles of 14 day old rats, according to the techinques already decribed (A. Fabbri, et al. - Opiate receptors are present in the rat testis: Identification and localization on Sertoli cells. Endocrinology 117: 2544-2546, 1985). Briefly, the procedure consisted in the separation of the tubular compartment from the Leydig cells by means of enzymatic dispersion with collagenase and subsequent treatment of the tubules with trypsin and collagenase. The separated cells were applied to 48 well Costar plates at the concentration of approximately 100,000 cells per well. The cells were allowed to adhere to the bottom of the wells for 24 hours in a metabolic incubator at 34°C, in 95% O_2/5% CO_2 The cells were then washed and incubated in TC 199 10 mM TRIS medium in the presence of iodine labelled GRF-44 at the concentration of 10^{-11}M for 60 minutes at 4°C in the presence or absence of 10^{-6} M cold (unlabelled) human GRF-44. Then the cells were washed 3 times with PBS + 0.5% BSA at 4°C; the radio-activity bound to the cells was then solubilized with 0.1 N NaOH and read in a gammacounter.

2. RESULTS

Immunoreactivity to GRF

Immunoreactivity values for GRF in the examined biological fluids were the following:
Follicular fluid = 60 pg/ml
Seminal fluid = 140 pg/ml
Leydig rat cell supernatant = 100 pg/ml.

Binding Sites on the Sertoli Cells

The results obtained showed the presence of specific binding of GRF to Sertoli cells equal to 2.5 fmoles of peptide/70 mcg of protein (about 40 fmoles/mg of protein).

3. CONCLUSIONS

These results indicate that:

1. immunoreactivity to GRF is present both in human follicular and seminal fluid;
2. immunoreactivity to GRF is present in the supernatant of Leydig rat cells and its binding sites are located on the Sertoli cells of the same animal species.

It can therefore be hypothesised that positive clinical effects on follicle-genesis and spermatogenesis could have been due to a direct action of the peptide at ovarian and testicular level, always considering the possibility of an effect mediated by other growth factors.

CLINICAL STUDIES

Treatment of female infertility.

In a clinical study carried out in conformity with the present invention women, both with normal follicle-genesis (as control) and follicle-genesis deficiency were treated.

The study consisted in echographic and hormonal monitoring of follicle-genesis during which the volume of the follicle in maturative growth and the plasma levels of 17-beta estradiol and 17-alpha OH progesterone were recorded.

In the controls with normal follicle-genesis, 2 monitorings of the follicle-genesis were carried out in accordance with the above mentioned procedures: the first verified the basal situation and the second was under administration of GRF-29 at the dosage of 100 or 150 mcg/day i.v. from the 1st to the 7th day of the cycle.

In patients with follicle-genesis deficiency, monitorings of the follicle-genesis were carried out: without any medicinal administration (as control); under administration of GRF-29 at the dosage of 100 or 150 mcg/day i.v. from the 1st to the 7th day of the cycle; under administration of FSH (METRODIN(R)) at the dosage of 75 or 150 I.U./day i.m. for 7 days; under the combined administration of FSH + GRF at the above dosage.

Case 1 (C.R.)

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Age 21 - Normal follicle-genesis - Control.

The first cycle of observation (basal) shows the presence of follicular activation and evidence of a dominant follicle between the 14th and the 15th day of the cycle, with ovulation between the 17th and the 18th day.

Day of cycle	Follicle diameter	17 beta E2	17 alpha OH P
1	absent	58	748
2	absent	62	840
3	5 mm	59	790
4	5 mm	64	864
5	5 mm	71	854
6	5 mm	69	843
7	5 mm	74	860
8	5 mm	78	890
9	6 mm	82	910
10	7 mm	84	896
11	7 mm	86	930
12	7 mm	88	915
13	10 mm right	94	913
14	11 mm right	112	980
15	15 mm right	127	1040
16	18 mm right	224	1470
17	20 mm right	196	1560
18	DEHISCENCE	136	1680

The second cycle of observation, carried out under therapy with GRF-29 (100 mcg/day i.v.), shows the presence of follicular activation and evidence of a dominant follicle between the 13th and the 14th day of the cycle, with ovulation between the 15th and 16th day.

Day of cycle	Follicle diameter	17 beta E2	17 alpha OH P
1	absent	43	470
2	absent	63	440
3	absent	58	530
4	5 mm	51	580
5	5 mm	49	550
6	5 mm	53	640
7	5 mm	48	620
8	5 mm	68	613

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9	5 mm	5	686
10	7 mm	74	694
11	9 mm	86	769
12	11 mm left	92	680
13	16 mm left	114	860
14	19 mm left	286	960
15	22 mm left	176	1214
16	DEHISCENCE		

Case 2 (S.A.)

Age 24 - Normal follicle-genesis - Control.

Basal observation:

Day of cycle	Follicle diameter	17 beta E2	17 alpha OH P
1	5 mm	57	326
2	5 mm	72	382
3	5 mm	61	358
4	5 mm	76	287
5	5 mm	74	269
6	10 mm	78	346
7	10 mm	73	358
8	10 mm	81	425
9	12 mm	95	462
10	13 mm	103	592
11	14 mm	112	588
12	16 mm	187	786
13	18 mm	135	1242
14	20 mm	106	1182
15	DEHISCENCE		

Cycle of observation under GRF-29 150 mcg/day i.v. from the 1st to the 7th day of the cycle:

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Day of cycle	Follicle diameter	17 beta E2	17 alpha OH P
1	5 mm	46	282
2	5 mm	43	198
3	5 mm	48	364
4	5 mm	62	372
5	5 mm	57	296
6	5 mm	68	318
7	7 mm	71	351
8	7 mm	82	370
9	10 mm	84	432
10	10 mm	86	445
11	12 mm	92	526
12	14 mm	114	788
13	17 mm	126	1128
14	20 mm	232	1246
15	20 mm	174	1751
16	DEHISCENCE		

Case 3 (C.A.)

Age 34 - Patient.

Four years of primary infertility due to follicle-genesis and ovulatory deficiency. Previous echographic and bio-chemical studies, and inductive therapy with clomiphene citrate + ethynylestradiol and/or gonadotrophins, show the presence of a multi-follicular activation without the possibility of evolution to dominance, but with a tendency to luteinization.
The first cycle of observation showed the absence of echographic signs of activation and follicular dominance and the presence of bio-chemical signs of ovarian activation.

Basal observation:

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Day of cycle	Follicle diameter	17 beta E2	17 alpha OH P
1	absent	74	140
2	absent	60	176
3	absent	48	103
4	absent	61	118
5	absent	42	123
6	absent	64	146
7	absent	96	191
8	absent	98	178
9	absent	120	196
10	absent	137	436
11	absent	162	764
12	absent	139	836
13	absent	87	578
14	absent	42	798
15	absent	57	1032
16	absent	60	2263
17	absent	64	2023
18	absent	96	876
19	absent	71	732
20	absent	42	876
21	absent	48	742
22	absent	38	936
23	absent	65	878
24	absent	72	804
25	absent	66	972

Menstrual flow occurred spontaneously on the 35th day of the cycle.

The second cycle of study, carried out under administration of GRF-29 (150 mcg/day i.v.), shows the presence of follicular activation, dominance and dehiscence. Taking into account the dimensions reached by the follicle, it may not be excluded that the disappearance of echographic observation after the 16th day of the cycle could be due to involution. Bio-chemical data testify the presence of ovulation in this cycle of observations.

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Day of cycle	Follicle diameter	17 beta E2	17 alpha OH P
1	absent	21	426
2	absent	24	386
3	5 mm	78	147
4	5 mm	42	220
5	7 mm	60	338
6	7 mm	30	470
7	7 mm	72	476
8	7 mm	81	512
9	7 mm	24	338
10	11 mm	86	780
11	11 mm	144	764
12	11 mm	198	940
13	12 mm	176	912
14	15 mm	166	560
15	16 mm	144	1215
16	18 mm	136	2131
17	DEHISCENCE		

The third cycle of study was carried out under administration of FSH (Metrodin) at the dosage of 150 I.U. from the 1st to the 7th day of the cycle. In this case echographic and bio-chemical signs of ovarian activation without follicular dominance were recorded.

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Day of cycle	Follicle diameter	17 beta E2	17 alpha OH P
1	absent	42	161
2	absent	54	191
3	5 mm	58	176
4	5 mm	52	182
5	5 mm	87	196
6	5 mm	132	212
7	5 mm	102	232
8	5 mm	112	312
9	5 mm	114	264
10	5 mm	136	287
11	5 mm	102	380
12	5 mm	164	412
13	5 mm	198	397
14	5 mm	86	514
15	5 mm	78	498
16	5 mm	36	780
17	5 mm	89	960
18	5 mm	86	1020
19	5 mm	98	1120
20	5 mm	107	1814
21	5 mm	54	1718
22	5 mm	62	1920
23	5 mm	67	1470
24	5 mm	65	1296
25	5 mm	44	1540

Menstrual flow re-occurred on the 26th day of the cycle.

The 4th cycle of study was carried out under administration of GRF-29 (150 mcg/day i.v.) + Metrodin(R) (150 I.U./day i.m.) from the 1st to the 7th day of the cycle. During this cycle it was possible to observe ovarian activation, follicular dominance and dehiscence on the 18th day of the cycle.

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Day of cycle	Follicle diameter	17 beta E2	17 alpha OH P
1	absent	52	291
2	absent	58	276
3	absent	72	364
4	5 mm	78	386
5	5 mm	86	355
6	7 mm	92	312
7	7 mm	112	296
8	7 mm	136	416
9	10 mm	128	492
10	10 mm	182	512
11	12 mm	214	980
12	14 mm	176	2050
13	16 mm	187	2986
14	16 mm	86	2250
15	18 mm	92	2322
16	20 mm	95	2435
17	20 mm	106	2679
18	DEHISCENCE		

Case 4 (F.L.)

Age 28 - Patient.

Patient suffering from primary couple sterility due to follicle-genesis deficiency. Previous studies demonstrated the absence of follicular maturation. By carrying out a stimulating therapy with clomiphene citrate or gonadotrophins it was possible in the past to demonstrate the presence of follicle-genesis activation and follicle dominance, not followed, however, by ovulation.
The first cycle of observation, carried out without any therapy, shows the absence of follicular activation and dominance after 25 days of study.

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Day of cycle	Follicle diameter	17 beta E2	17 alpha OH P
1	absent	74	670
2	absent	87	976
3	absent	79	986
4	absent	83	890
5	absent	56	760
6	absent	78	857
7	absent	58	912
8	absent	67	911
9	absent	73	892
10	absent	84	695
11	absent	103	785
12	absent	97	775
13	absent	88	875
14	absent	89	798
15	absent	99	823
16	absent	112	811
17	absent	74	820
18	absent	86	756
19	absent	62	810
20	absent	70	769
21	absent	66	758
22	absent	72	814
23	absent	81	832
24	absent	58	906
25	absent	65	876

Menstrual flow occurred spontaneously on the 38th day.

The second cycle of study carried out under administration of GRF-29 (100 mcg/day i.v.) demonstrates the absence of ovulation but follicle-genesis was activated.

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Day of cycle	Follicle diameter	17 beta E2	17 alpha OH P
1	absent	54	689
2	absent	56	740
3	absent	53	640
4	absent	64	678
5	absent	51	721
6	absent	58	707
7	5 mm	53	814
8	5 mm	62	821
9	5 mm	60	824
10	7 mm	67	811
11	7 mm	72	854
12	7 mm	69	814
13	7 mm	87	863
14	10 mm	98	798
15	10 mm	92	902
16	10 mm	74	904
17	10 mm	86	870
18	10 mm	94	986
19	10 mm	98	965
20	10 mm	87	935
21	10 mm	83	879
22	10 mm	81	954
23	10 mm	78	906
24	10 mm	90	887
25	10 mm	97	808

Menstrual flow occurred spontaneously on the 33rd day.

The third cycle of the study was carried out under administration of FSH (Metrodin(R)) in ampoules of 75 IU/day i.m. from the 1st to the 7th day of the cycle.

Also in this case ovarian activation was demonstrated but correct follicle-genesis and ovulation were absent.

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Day of cycle	Follicle diameter	17 beta E2	17 alpha OH P
1	absent	55	760
2	absent	47	789
3	absent	49	754
4	5 mm	60	810
5	5 mm	58	796
6	7 mm	46	840
7	7 mm	65	786
8	7 mm	90	441
9	7 mm	87	534
10	7 mm	88	675
11	7 mm	63	632
12	7 mm	68	652
13	7 mm	66	678
14	7 mm	78	691
15	7 mm	102	735
16	7 mm	96	709
17	7 mm	113	809
18	7 mm	95	807
19	absent	104	812
20	absent	86	811

During the evening of the 20th day, menstrual flow re-occurred.

The fourth cycle of study was carried out under administration of GRF-29 100 mcg/day + Metrodin(R) 75 IU/day via i.v. e i.m. routes respectively from the 1st to the 7th day of the cycle. During this period of observation it was possible to observe a maturative evolution of the follicle with ovulation between the 18th and the 19th day of the cycle.

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Day of cycle	Follicle diameter	17 beta E2	17 alpha OH P
1	absent	42	327
2	absent	43	543
3	5 mm	44	436
4	5 mm	48	438
5	5 mm	42	395
6	5 mm	54	540
7	5 mm	43	365
8	9 mm left	126	654
9	9 mm left	84	675
10	12 mm left	72	643
11	13 mm left	96	635
12	16 mm left	108	547
13	16 mm left	78	673
14	19 mm left	99	768
15	20 mm left	150	987
16	22 mm left	165	1237
17	22 mm left	145	1570
18	22 mm left	126	1768
19	DEHISCENCE		

Case 5 (A.V.)

Age 30 - Patient.

This patient was included in the study because of primary couple sterility due to follicle maturation deficiency, anovulation and follicular luteinization. Cycles of stimulating therapy with clomiphene citrate and gonadotrophins had been carried out without resolving the clinical situation.
The first cycle of observation, carried out without any therapy, shows the presence of a delayed ovarian activation, of anovulation and follicular luteinization.

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Day of cycle	Follicle diameter	17 beta E2	17 alpha OH P
1	absent	36	219
2	absent	41	228
3	absent	32	217
4	absent	51	307
5	absent	45	280
6	absent	38	275
7	absent	37	298
8	5 mm	32	345
9	5 mm	48	318
10	8 mm	45	321
11	8 mm	42	315
12	8 mm	46	324
13	8 mm	36	322
14	8 mm	38	357
15	12,1 mm right	87	432
16	13 mm right	125	456
17	14,6 mm right	240	483
18	15,9 mm right	144	632
19	20,3 mm right	222	608
20	20 mm right	162	634
21	21 mm right	96	687
22	30,3 mm right	109	623

Observations on the 22nd day of the cycle revealed the presence of irregularities in the wall of the dominant follicle, showing inner luteinization echoes. Suspension of monitoring was therefore decided. Menstrual flow re-occurred on the 35th day. An echographic study carried out during the menstrual flow demonstrated the disappearance of the follicular formation.

The second cycle of study was carried out under administration of FSH (Metrodin(R)) 75 I.U./day i.m. from the 1st to the 7th day of the cycle. It showed a substantial persistence of the situation observed during the cycle of control, except for more precocious ovarian activation.

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Day of cycle	Follicle diameter	17 beta E2	17 alpha OH P
1	absent	56	436
2	absent	58	412
3	absent	45	408
4	absent	57	367
5	6 mm	52	418
6	6 mm	65	436
7	8 mm	60	562
8	8 mm	68	652
9	8 mm	78	609
10	12 mm left	108	658
11	12,7 mm left	125	634
12	14 mm left	234	676
13	14,3 mm left	248	657
14	14 mm left	167	768
15	16,5 mm left	225	789
16	17,6 mm left	161	750
17	21,5 mm left	287	806
18	26,8 mm left	308	805
19	26,5 mm left	237	865
20	26,5 mm left	143	906
21	26,3 mm left	128	987

Observations on the 21st day of the cycle demonstrated clear inner luteinization-like echoes of the dominant follicle. It was therefore decided to suspend the study. Menstrual flow re-occurred spontaneously on the 26th day of the cycle.
An echographic check carried out during the menstrual flow demonstrated the disappearance of the follicular formation during this cycle.

The third cycle of study was carried out under administration of 100 mcg/day i.v. of GRF-29 + 75 I.U. of FSH (Metrodin(R))/day i.m. from the 1st to the 7th day of the cycle. During this period it was possible to observe a better maturative evolution of the follicle with ovulation between the 16th and 17th day of the cycle.

BNSDOCID: <EP___0300982A2_I_>

Day of cycle	Follicle diameter	17 beta E2	17 alpha OH P
1	absent	36	287
2	absent	34	280
3	absent	46	310
4	6 mm	48	298
5	8 mm	57	346
6	8 mm	58	320
7	8 mm	50	318
8	8 mm	68	457
9	10 mm	102	505
10	12 mm right	126	537
11	14 mm right	156	518
12	16 mm right	142	609
13	20 mm right	245	1027
14	21 mm right	287	1124
15	21 mm right	157	1245
16	21 mm right	134	1347
17	DEHISCENCE		

Menstrual flow re-occurred during the 29th day of the cycle.

Case 6 (C.P.)

Age 32 - Patient.

Eight years of primary sterility. Diagnosis of micropolycistic ovary.
Absence of follicle-genesis activation, also under gonadotrophin induction. The patient under observation had been ovariectomized on the left side.
During the first cycle of observation, without any therapy, it was possible to observe echographic, but not bio-chemical, signs of ovarian activation and absence of follicular dominance.

BNSDOCID: <EP___0300982A2_I_>

Day of cycle	Follicle diameter	17 beta E2	17 alpha OH P
1	absent	36	402
3	absent	12	386
4	absent	47	414
5	absent	72	428
6	absent	65	472
7	absent	49	518
8	absent	58	464
9	absent	72	532
10	5 mm	32	412
11	5 mm	38	651
12	5 mm	46	646
13	5 mm	41	807
14	5 mm	71	687
15	5 mm	76	792
16	5 mm	49	815
17	5 mm	55	842
18	5 mm	47	812
19	5 mm	49	814
20	absent	67	1020
21	absent	47	819
22	absent	76	1112
23	absent	59	1046
24	absent	67	1100
25	absent	61	976

Menstrual flow re-occurred spontaneously on the 37th day of the cycle. It was very scarce, and lasted for 2 days (anomalous flow).

The second cycle of observation was carried out under administration of GRF-29 150 mcg/day i.v. from the 1st to the 7th day of the cycle.

A good ovarian activation was observed, followed by the growth of two follicles (within a micropolycistic ovary, as stated above), which presented an involution around the 17th day of the cycle.

BNSDOCID: <EP___0300982A2_I_>

Day of cycle	Follicle diameter	17 beta E2	17 alpha OH P
1	absent	28	176
2	absent	48	162
3	absent	24	382
4	5 mm	66	382
5	5 mm	78	264
6	7 mm	64	147
7	7 mm	79	173
8	9/7 mm	75	186
9	9/8 mm	78	191
10	12/8 mm	112	286
11	12/8 mm	120	367
12	12/8 mm	136	842
13	12/8 mm	90	940
14	12/8 mm	132	913
15	12/8 mm	138	942
16	12 mm	142	1012
17	INVOLUTION	112	1067

The third cycle of observation was carried out administering Metrodin(R) 150 I.U./day i.m. from the 1st to the 7th day of the cycle. Ovarian activation without follicular dominance was echographically and bio-chemically demonstrated.

BNSDOCID: <EP__0300982A2_I_>

Day of cycle	Follicle diameter	17 beta E2	17 alpha OH P
1	absent	26	146
2	absent	28	186
3	absent	52	216
4	absent	84	342
5	absent	56	287
6	5 mm	83	298
7	5 mm	108	188
8	7 mm	112	214
9	7 mm	138	236
10	7 mm	142	287
11	7 mm	126	312
12	7 mm	116	335
13	7 mm	126	288
14	7 mm	114	300
15	7 mm	118	306
16	7 mm	87	297
17	7 mm	89	480
18	INVOLUTION		

Menstrual flow re-occurred on the 21st day of the cycle.

The fourth observation was carried out administering GRF-29 150 mcg/day i.v. + FSH (Metrodin(R)) 150 I.U./day i.m. from the 1st to the 7th day of the cycle. Ovarian activation and follicular dominance were observed, with dehiscence around the 14th day of the cycle.

BNSDOCID: <EP___0300982A2_I_>

Day of cycle	Follicle diameter	17 beta E2	17 alpha OH P
1	absent	26	186
2	absent	48	296
3	absent	51	217
4	5 mm	76	368
5	7 mm	72	412
6	10 mm	94	615
7	10 mm	102	627
8	12 mm	112	742
9	15 mm	126	762
10	16 mm	138	713
11	18 mm	241	816
12	19 mm	312	1120
13	24 mm	106	2400
14	DEHISCENCE		

Case 7 (E.L.)

Age 37 - Patient

Primary sterility. Absence of ovarian activation, also under administration of high dosages of gonadotrophins. The patient was in secondary amenorrhea and presented a flow only after administration of progesterone. Prior to commencement of the study, menstrual flow was induced by administering Gestone(R) 100 mg i.m. The first cycle of observation carried out without any therapy confirmed the absence of echographic and bio-chemical notes on ovarian activation.

BNSDOCID: <EP___0300982A2_I_>

Day of cycle	Follicle diameter	17 beta E2	17 alpha OH P
1	absent	21	182
2	absent	28	206
3	absent	32	164
4	absent	36	106
5	absent	31	118
6	absent	30	146
7	absent	32	172
8	absent	34	181
9	absent	28	196
10	absent	26	172
11	absent	21	231
12	absent	37	356
13	absent	27	328
14	absent	35	318
15	absent	37	372
16	absent	32	310
17	absent	30	336
18	absent	27	327
19	absent	21	311
20	absent	26	286
21	absent	42	215
22	absent	41	217
23	absent	38	204
24	absent	36	236
25	absent	51	287

On the 45th day of the cycle, the flow not having re-occurred, menstruation was induced by administering Gestone(R) 100 mg, i.m.

The second cycle of observation was carried out administering GRF-29 150 mcg/day i.v. from the 1st to the 7th day of the cycle. The presence of echographic notes of ovarian activation, but absence of follicular dominance, was recorded.

BNSDOCID: <EP___0300982A2_I_>

Day of cycle	Follicle diameter	17 beta E2	17 alpha OH P
1	absent	22	182
2	absent	23	170
3	absent	28	212
4	absent	31	186
5	5 mm	36	214
6	5 mm	32	182
7	5 mm	38	116
8	7 mm	30	86
9	7 mm	35	214
10	7 mm	41	213
11	7 mm	32	218
12	7 mm	46	236
13	7 mm	48	232
14	7 mm	51	214
15	7 mm	52	182
16	INVOLUTION		

Menstrual flow was induced by administering Gestone(R) 100 mg i.m. on the 45th day of the cycle. The third cycle of observation was carried out under administration of FSH (Metrodin(R)), 150 U.I./day i.m. from the 1st to the 7th day of the cycle. It was possible to observe echographic, but not bio-chemical, signs of ovarian activation and the absence of follicular dominance.

BNSDOCID: <EP___0300982A2_I_>

Day of cycle	Follicle diameter	17 beta E2	17 alpha OH P
1	absent	36	112
2	absent	38	110
3	absent	46	114
4	5 mm	42	162
5	10 mm	44	176
6	10 mm	48	182
7	10 mm	56	196
8	10 mm	58	182
9	10 mm	64	187
10	10 mm	68	172
11	10 mm	52	198
12	10 mm	56	176
13	10 mm	58	187
14	10 mm	55	195
15	10 mm	46	164
16	10 mm	58	156
17	10 mm	64	187
18	10 mm	72	209
19	10 mm	38	212
20	10 mm	36	214
21	10 mm	28	207
22	10 mm	32	286
23	10 mm	41	211
24	10 mm	32	189
25	10 mm	46	214

Menstrual flow was induced by Gestone(R) 100 mg on the 45th day of the cycle.

The fourth cycle of observation was carried out under administration of GRF-29 150 mcg/day i.v. + Metrodin(R) 150 I.U./day i.m. from the 1st to the 7th day of the cycle. It was possible to demonstrate the presence of echographic and bio-chemical signs of follicular activation, dominance and dehiscence.

BNSDOCID: <EP___0300982A2_I_>

Day of cycle	Follicle diameter	17 beta E2	17 alpha OH P
1	absent	31	386
2	absent	38	394
3	absent	24	470
4	5 mm	34	570
5	7 mm	76	640
6	7 mm	132	720
7	7 mm	139	550
8	10 mm	104	420
9	10 mm	114	436
10	10 mm	188	482
11	13 mm	186	560
12	14 mm	282	630
13	16 mm	308	985
14	20 mm	304	1760
15	22 mm	346	3280
16	DEHISCENCE		

These results demonstrate that administration of GRF-29 during the first phase of the cycle facilitates follicle growth and anticipates, or even induces, ovulation (dehiscence). Furthermore, GRF was capable of coadjuvating, always at follicular level, the effect of FSH in patients with documented anovulation. In these latter cases, treatment with GRF in combination with FSH always caused efficacious follicle growth up until ovulation.

Treatment of male infertility

Patients suffering spermatogenesis deficiences of various entity with oligospermia or azoospermia, selected according to the criteria of the protocol, were treated for a period of three months with GRF-29 at the dosage of 5 mcg/kg s.c. 3 times a week.

In conformity with the criteria of inclusion in the protocol, all the patients ranged between 25 and 40 years, with normal or above average gonadotrophin levels, and did not present inflammatory processes or other diseases which could have interfered with the seminal parameters. Testicular biopsy showed an arrest of spermatogenesis at spermatid level in the absence of peritubular fibrosis.

The first control was carried out after 45 days from commencement of therapy, and the second control after 3 months. The tests showed an improvement of the seminal parameters, and especially an increase in the number of spermatozoa and their motility, from which the efficacy of the therapy can be deduced.

The above described treatment was carried out by using GRF alone, in consideration of the normal or above average gonadotrophin levels, based on the criteria of inclusion in the protocol. It falls within the scope of the present invention to use GRF to amplify the effect of gonadotrophins in all cases of spermatogenesis deficiency, due to either testicular pathologies or gonadotrophinic secretion deficiency (hypogonadotrophic hypogonadism).

Although the clinical studies reported above were carried out by using FSH as gonadotrophin, it is evident that similar results can be obtain d by administ ring Menotrophin (HMG) or any other preparation containing FSH.

Similarly, the use of GRF-44, or other molecules having analogous activity, in place of the GRF-29 which was used in the above reported clinical studies, can be considered as equivalent.

BNSDOCID: <EP___0300982A2_I_>

Claims

1) Use of a Somatotrophin releasing-factor to produce a pharmaceutical preparation for the treatment of infertility.

2) Use according to claim 1 wherein the pharmaceutical preparation for the treatment of infertility includes a pharmacologically efficacious quantity of a Somatotrophin releasing-factor and one or more excipients.

3) Use according to Claims 1 and 2 in which the Somatotrophin releasing-factor is selected from any of GRF-29, GRF-37, GRF-40, GRF-44, GRF-NH_2-44 or mixtures thereof.

4) Use according to claims 1 to 3 in which the pharmaceutical preparation is used simultaneously, sequentially or separately with a pharmacologically efficacious quantity of follicle stimulating hormone.

5) Use according to claim 4 wherein the follicle-stimulating hormone is in the form of a pharmaceutical preparation.

6) Product containing a somatotrophin releasing-factor and follicle stimulating hormone for use as a medicament.

7) Product according to claim 6 for use in the treatment of infertility.

8) Product according to claim 6 and 7 obtainable by simultaneous, separate or sequential use of somatotrophin releasing-factor and follicle stimulating hormone.

9) Product according to claims 6 to 8 in which the Somatotrophin releasing-factor is selected from any of GRF-29, GRF-37, GRF-40, GRF-44, GRF-NH_2-44 or mixtures thereof.

10) Product according to claims 6 to 9 in which the follicle stimulating hormone is in the form of Menotrophin, Urofollitrophin or mixtures thereof.

11) Pharmaceutical composition containing a Somatotrophin releasing-factor and follicle-stimulating hormone, in the presence of one or more pharmaceutically acceptable excipients, for the simultaneous, separate or sequential use of its active components in the treatment of infertility.

12) Pharmaceutical composition according to Claim 11 containing a somatotrophin releasing-factor selected from any of GRF-29, GRF-37, GRF-40, GRF-44, GRF-NH_2-44 or mixtures thereof and follicle-stimulating hormone in the presence of one or more pharmaceutically acceptable excipients.

13) Pharmaceutical composition according to Claims 11 and 12 wherein the follicle-stimulating hormone is selected from either Menotrophin or Urofollitrophin or mixtures thereof.

14) Pharmaceutical composition according to claims 11 and 13 in which the Somatotrophin releasing-factor is GRF-29.

15) Pharmaceutical composition according to claims 11 to 14 obtainable by simultaneous, separate or sequential use of a pharmacologically efficacious quantity of somatotrophin releasing-factor with a pharmacologically efficacious quantity of follicle stimulating hormone.

16) Pharmaceutical composition according to claim 15 wherein the somatotrophin releasing-factor is in the form of a pharmaceutical preparation as defined in claims 1 to 3.

17) Pharmaceutical composition according to claims 15 and 16 wherein the follicle stimulating hormone is in the form of a pharmaceutical preparation.

18) Pharmaceutical composition according to claim 17 wherein the follicle-stimulating hormone is selected from either Menotrophin or Urofollitrophin or mixtures thereof.

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(54) Treatment of infertility.

(57) The use of somatotrophin releasing factor (GRF) is disclosed for the treatment of infertility. Also disclosed for such treatment is the use of somatotrophin releasing factor along with gonadotrophins, particularly follicle stimulating hormone (FSH) or preparations containing FSH. Pharmaceutical preparations containing somatotrophin releasing factor, including preparations containing both somatotrophin releasing factor and follicle stimulating hormone, are also disclosed.

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EUROPEAN SEARCH REPORT

Application Number

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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
A	BIOLOGICAL ABSTRACTS, vol. 84, no. 12, 1987, abstract no. 117704, Biological Abstracts, Inc., Philadelphia, PA, US; R.C. GAILLARD: "The hypothalamic factors: Recent diagnostic and therapeutic advances", & SCHWEIZ MED WOCHENSCHR 117(34): 1270-1277. 1987 ---		A 61 K 37/43
A	PROC. NATL. ACAD. SCI. USA, vol. 79, December 1982, pages 7909-7913, US; P. BRAZEAU et al.: "Growth hormone releasing factor, somatocrinin, releases pituitary growth hormone in vitro" -----		
			TECHNICAL FIELDS SEARCHED (Int. Cl.4)
			A 61 K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	12-10-1989	TURMO Y BLANCO C.E.

CATEGORY OF CITED DOCUMENTS

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